New treatments for severe treatment-resistant asthma: targeting the right patient

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Guidelines for asthma management focus on the use of combination inhaled treatment with corticosteroids and longacting β-agonists for symptomatic asthma. In more severe disease, other drugs such as leukotriene blockers and slow-release oral theophylline are added, with oral corticosteroids and anti-immunoglobulin E treatment with omalizumab for the most severe cases of asthma. Once-daily longacting β-agonists and inhaled corticosteroids are being developed. Longacting muscarinic antagonists might also provide additive benefit. New approaches are needed for the treatment of severe asthma, but patients need to be endotyped so that they can be directed for specific treatments. This Review focuses on the role of eosinophilic and neutrophilic inflammation, the attributes of chronic airflow obstruction, and the notion of corticosteroid insensitivity because potential targets for treatment have started to emerge from such analyses. How the best phenotypic or even better, the best endotypic responder with each new treatment, can be established will also be discussed. Newer treatments for asthma will emerge from better endotyping, leading to personalised medicine in asthma.

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For more on **GINA guidelines** see http://www.ginasthma.org/documents/1/Pocket-Guide-for-Asthma-Management-and-Prevention

Introduction

In the past 20 years, combined use of a bronchodilator, a longacting β-adrenergic agonist, and anti-inflammatory corticosteroids, by the inhaled route has become the most effective mainstay treatment of asthma. This treatment forms the backbone of the guidelines of the Global Initiative for Asthma (GINA), in which inhaled combination longacting β-adrenergic agonist and corticosteroid are used at steps three and above for maintenance of control of asthma. The efficacy of such combination treatments has been assessed in studies of adult patients with asthma who are on low to high doses of inhaled corticosteroids; the addition of a longacting β-adrenergic agonist reduced the occurrence of exacerbations needing oral corticosteroids, improved lung function (forced expiratory volume in 1 s [FEV_1]), and decreased the need for rescue shortacting β-agonists.[1] At the higher step four of the GINA treatment guidelines, other treatments such as slow-release theophylline and leukotriene inhibitors can be added in cases of poor control despite the use of combination longacting β-adrenergic agonist and inhaled corticosteroid treatment.[2] In a study of the effect of combined treatment with a longacting β-adrenergic agonist and an inhaled corticosteroid, asthma control (defined by GINA guidelines) was achieved in only 68% of patients with varying severity of disease, with the least number in the most severe group,[3] suggesting that even treatment at the maximum doses allowable is not effective in all patients with asthma. At step five, representing the most severe cases of asthma, the addition of oral treatment with corticosteroids is advocated. In the past 5 years, a new class of treatment, an anti-immunoglobulin E humanised monoclonal antibody, has been introduced to treat severe allergic asthma at this step five. Although great progress has been made in establishing treatment guidelines for asthma, a great need for new treatments for asthma still exists because an estimated 5–10% of patients with asthma are refractory to available treatments. Such patients have been labelled as having severe asthma or refractory-resistant asthma.[4,5] The nature of these unmet needs are being defined, and patients who do not respond to treatment remain a substantial part of the asthma burden. More specifically, targeted novel treatments are needed in this group of patients. Asthma cannot be regarded as one disease, but as an umbrella term that embraces a collection of several different phenotypes that could be mediated by different pathways. The clinical heterogeneity of asthma is being recognised in patients, especially in those with severe asthma, and clinical characteristics that specify subgroups or phenotypes of asthma that are caused by different mechanisms are

Key Messages

- Asthma guidelines emphasise the use of combination treatment of inhaled corticosteroids and longacting β-adrenergic agonists as the core treatment, with the addition of other controllers including oral corticosteroids in more severe asthma.
- Asthma is regarded as a heterogeneous disorder with distinct phenotypes. Specific features pertaining to severe asthma include chronic airflow obstruction, eosinophilic and neutrophilic asthma, corticosteroid insensitivity, and recurrent exacerbations.
- Specific treatments for asthma are unlikely to benefit all patients with asthma, but more likely target specific phenotypes. Biomarkers to predict responsiveness to these treatments should be used.
- Anti-immunoglobulin E treatment with omalizumab has been introduced for allergic severe asthma, and concentrations of nitric oxide in exhaled breath, blood eosinophil counts, and serum periostin concentrations are associated with a good therapeutic response.
- Anti-interleukin-5 antibody treatment is effective in reducing exacerbation rates in patients on high-dose corticosteroids with sputum eosinophilia.
- Blocking interleukin 13 with an anti-interleukin-13 antibody is associated with an improvement in FEV_1 in patients with asthma who have high concentrations of exhaled nitric oxide and serum periostin, a marker of T-helper-2 cell activation.
- Treatments targeted at neutrophilic asthma and steroid insensitivity are needed.
- Endotyping patients with severe asthma with links to pathophysiological mechanisms will lead to a more precise and rational way of getting specific treatments to the individual patient, one step towards personalised medicine.

being investigated. Focus on a specific target in a well defined phenotype of asthma is more likely to lead to a more successful treatment outcome than in an unphenotyped group. The idea of one-treatment-fits-all, although applicable to a patient presenting for the first time with asthma, cannot be applied to a patient with severe asthma.

This Review outlines new treatments and potentially emerging treatments, progress in asthma phenotyping, and some of the challenges that lay ahead in both phenotyping and finding new treatments. Although this Review focuses on adults with asthma because most of the new treatments are being tested in this group, concomitant studies of asthma in children are also discussed.

Asthma phenotyping and endotyping

Hierarchical cluster analysis has identified clusters of patients with preserved lung function and little disease activity, those with early onset disease with atopic background, and a more severe cluster associated with adult onset and active disease.[6–8] New clinical groups have been described—eg, obese uncontrolled and obese well controlled.[8] Differences between these groups exist with regard to age of asthma onset, measures of asthma symptoms and control, exhaled nitric oxide concentration, and airway hyperresponsiveness.[9] Clusters of severe asthma, defined as clusters four and five of the adult severe asthma research programme cohort, were on high level treatment at steps four and five and associated with severe airflow obstruction, similar to steps four and five of the GINA guidelines.[10] Although such analyses provide some idea of disease severity, more useful associations will be found with the inclusion of biological biomarkers, which might inform on potential pathophysiological mechanisms and, ultimately, specificity of response to treatments.[11,12] Research into severe asthma has led to the identification of pathophysiological characteristics that might become part of specific phenotypes (panel). Although much study has been done on the T-helper-2 pathways,[13] non-T-helper-2 pathways are also important in some types of asthma (figure 1). The addition of known pathophysiological mechanisms into the phenotypic characterisation has been called endotyping.[14] In children, the clinical expression of severe asthma is highly variable and distinct severe asthma phenotypes are probably less well-defined in children than in adults.[7]

***Panel:* Extreme characteristics of severe asthma**

- Early onset of asthma in childhood versus late onset of asthma in adulthood
- Chronic airflow obstruction versus normal lung function
- Recurrent exacerbations versus occasional exacerbations
- Atopic with high serum immunoglobulin E versus non-atopic with normal serum immunoglobulin E
- Eosinophil versus non-eosinophil sputum
- T-helper-2 high versus T-helper-2 low
- Corticosteroid insensitive versus corticosteroid sensitive

Eosinophilic asthma

Sputum eosinophilia—defined as eosinophils present at 2% or more in sputum samples—is found in 36% of patients with asthma not on inhaled corticosteroid treatment and in 17% of patients given inhaled corticosteroids.[15] The addition of sputum eosinophil counts in a cluster analysis led to the identification of two clusters of severe asthma. One cluster was characterised by early onset, symptom-predominant disease but with minimum eosinophils, with a high prevalence of obesity, and female sex. The other cluster consisted of an eosinophilic inflammation-predominant group with few symptoms, late-onset disease, and an increased proportion of males with a high prevalence of rhinosinusitis, aspirin sensitivity, and exacerbations.[8] Measurement of sputum eosinophils could be used to guide adjustment of asthma treatments, resulting in improved asthma control with fewer exacerbations than the conventional use of symptoms or peak expiratory flow measurements.[16,17] Refractory patients with high sputum eosinophilia and recurrent exacerbations responded to specific anti-interleukin-5 monoclonal antibody treatment and had a reduced number of exacerbations.[18] Thus, patients with a subphenotype of severe asthma with recurrent exacerbations and sputum eosinophilia would benefit from anti-interleukin-5 treatments.[17,18] Although sputum eosinophilia was present in children with severe asthma, using it to guide management did not lead to a reduction in exacerbations or improvement in asthma control.[19] The table summarises the potential treatments available that target specific types of asthma including allergic asthma, eosinophilic asthma, neutrophilic asthma, chronic airflow obstruction, and recurrent exacerbations.

T-helper-2-high endotype

Individuals with mild to moderate asthma can be divided into T-helper-2-high and T-helper-2 low groups on the basis of mRNA expression in airway epithelial cell brushings of the interleukin-13-inducible genes, periostin, chloride channel regulator 1, and serpin peptidase inhibitor.[24] The T-helper-2 high patients had a specific type of asthma characterised by increased bronchial hyperresponsiveness, increased serum immunoglobulin E concentrations, increased blood and airway eosinophilia, subepithelial fibrosis, and airway mucin gene expression.[25] Furthermore, these patients responded well to inhaled corticosteroid treatment in terms of improvement in FEV_1, whereas those with a low T-helper-2 signature did not respond. Such endotyping might be useful in showing those patients who would respond well to inhaled corticosteroid treatment, although its association with eosinophilic asthma remains unclear and further refined phenotyping needs to be done. In

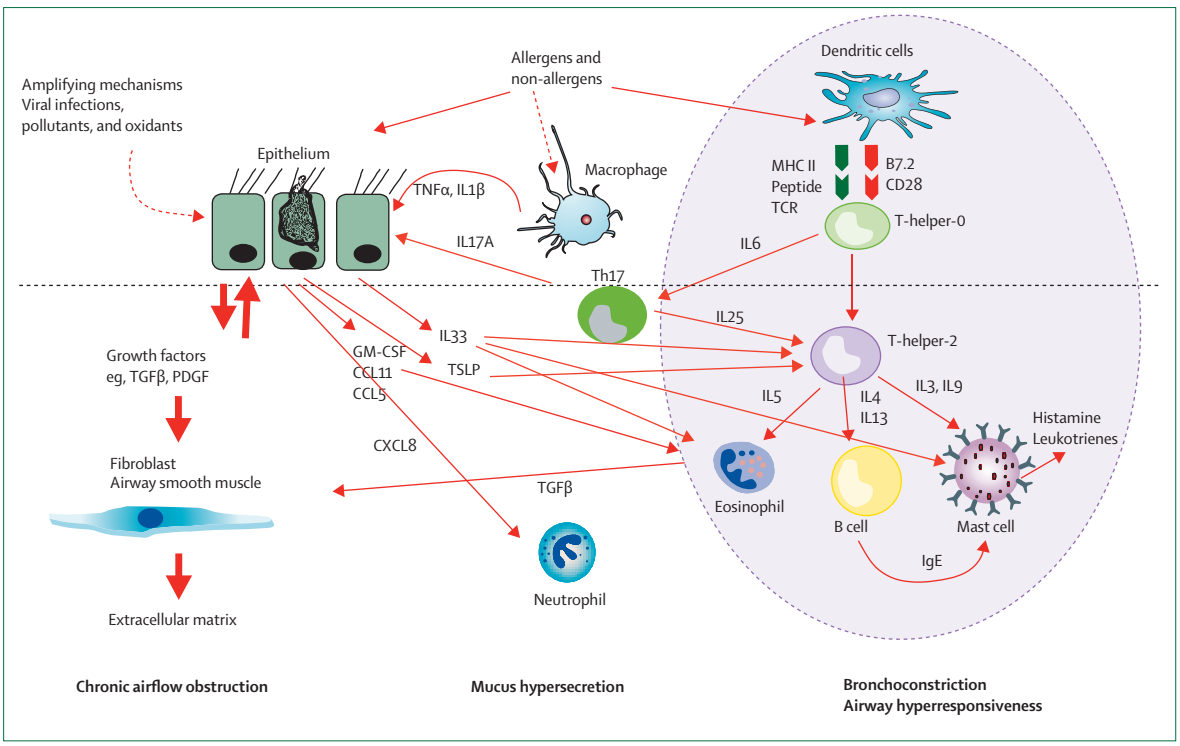

Figure 1: **Airway inflammation in asthma underlying chronic airflow obstruction, airway hyperresponsiveness, and mucus hypersecretion, focusing on T-helper-2 cytokines**

TNFα=tumour necrosis factor α. IL=interleukin. MHC=major histocompatibility complex. TCR= T cell receptor. IgE=immunoglobulin E. TGFβ=transforming growth factor β. PDGF=platelet-derived growth factor. GM-CSF=granulocyte macrophage-colony stimulating factor. CCL=C-C chemokine ligand. TSLP=thymic stromal lymphopoietin. CXCL=C-X-C chemokine ligand.

children, biomarkers such as increased concentrations of fractional exhaled nitric oxide (FeNO) and serum immunoglobulin E, which are indicators of processes driven by T-helper-2 cells, can be used to differentiate severe asthma from milder asthma.[26] However, in a study of bronchoalveolar lavage fluid in children with asthma, concentrations of T-helper-2 cytokines were very low although airway mucosal eosinophilia was present.[27] Therefore, the situation might be different in children.

Neutrophilic asthma

Non-eosinophilic asthma, consisting mostly of neutrophilic asthma, is more predominant in patients with mild to moderate asthma,[15] whereas neutrophilic asthma is predominant in patients with severe refractory asthma.[28,29] Baines and colleagues[30] found that genes in the interleukin 1 and tumour necrosis factor α (TNFα)/nuclear factor-κB pathways were overexpressed in cells recovered from induced sputum and associated with clinical parameters and neutrophilic airway inflammation. Patients with severe asthma with mixed neutrophilia and eosinophilia have worse lung function, increased frequency of daily wheeze, and increased health-care utilisation compared with patients with non-severe asthma.[28,31] The mechanisms behind these diverse inflammatory profiles are probably complex, but a neutrophilic response might be indicative of a disease mechanism that is not driven by T-helper-2 cells and most likely, non-steroid responsive asthma. Bacterial colonisation in the airways of patients with severe asthma could contribute to neutrophilic asthma[32,33] and has been associated with the defective phagocytosis of bacteria and apoptotic cells by macrophages.[34,35] These processes could contribute to the amount of oxidative stress in the airways and underlie corticosteroid insensitivity in patients with severe asthma. Oral corticosteroid treatment can contribute to neutrophilia to some degree.[36] T-helper-17 immune cells have been implicated as a cause of neutrophilia, with some supporting data from studies of severe asthma.[37] No clear data are available for the importance of neutrophilic inflammation in children.

The use of neutrophil counts in induced sputum to define neutrophilic inflammation is not ideal because neutrophils, unlike eosinophils, are a normal constituent of the cells retrieved in induced sputum, and the cutoff point suggesting an increase in neutrophil counts has not been defined. Other indicators of neutrophilic inflammation in the lungs need to be developed. Recent preliminary data suggest that the amount of hydrogen sulphide in induced sputum might be such an indicator, in addition to a possible measure of the degree of airflow obstruction.[38]

	Biomarkers	Specifically-targeted treatments
Allergic asthma[20]	High serum immunoglobulin E; atopy; high blood eosinophil	Anti-immunoglobulin E (omalizumab)
Eosinophilic asthma[21]	Recurrent exacerbations; sputum eosinophils; steroid-dependent asthma	Anti-interleukin-4 receptor α (dupilumab)
Neutrophilic asthma[22]	Sputum neutrophils	Macrolide antibiotics (azithromycin)
Chronic airflow obstruction[23]	Airway wall remodelling; low FEV_1; high serum periostin	Anti-interleukin-13 (lebrikizumab)
Recurrent exacerbations[17,18]	Sputum eosinophils; oral corticosteroid dose	Anti-interleukin-5 (mepolizumab)

FEV_1=forced expiratory volume in 1 s.

Table: **Phenotype-targeted treatments in asthma**

Chronic airflow obstruction

One characteristic of severe asthma is chronic airflow obstruction associated with air trapping,[39] which has been characterised on high resolution CT scanning and is secondary to obstruction of the small airways.[40,41] The degree of airflow obstruction has been linked to the degree of airway wall remodelling and inflammation.[42,43] The increased airway wall thickness in patients with severe asthma is associated with the pathologic changes of airway wall remodelling and with the degree of airflow obstruction.[44] The increased airway wall thickness constitutes an increase in airway smooth muscle mass and subepithelial fibrosis.[45] Patients with moderately severe asthma had a good bronchodilator response to an antibody to the T-helper-2 cytokine, interleukin 13,[46] especially those who had high serum concentrations of the biomarker periostin, which is stimulated by interleukin 13 and is associated with fibrosis.[47,48] Other biomarkers involved in the pathophysiology of the airway remodelling process need to be confirmed, but targeting the increase in airway smooth muscle mass and fibrosis through inhibition of the effects of growth factors such as TGFβ and PDGF might be necessary (figure 1).

Asthma exacerbations

An American Thoracic Society and a European Respiratory Society Task Force has defined asthma exacerbations as "events that require urgent action on the part of the patient and physician to prevent a serious outcome, such as hospitalisation or death from asthma".[49] A severe exacerbation is defined by events that need the use of systemic corticosteroids, an increase from a stable maintenance dose, or an event requiring hospitalisation or visit to accident and emergency because of asthma. A patient with asthma who has regular exacerbations is more likely to have comorbid factors such as severe sinus disease, gastro-oesophageal reflux, recurrent respiratory infections, and obstructive sleep apnoea.[50] The Severe Asthma Research Program cluster analysis reported that at least 54% of patients with severe asthma had three or more bursts of corticosteroid treatments to control exacerbations and that those patients with airflow obstruction had a higher exacerbation risk.[10] An association of increased risk of exacerbations with increased airway closure between exacerbations has been described;[51] air trapping is more often related to a history of intensive care visits or of mechanical ventilation.[40]

Fixed combination of an inhaled corticosteroid and a longacting β-adrenergic agonist is most effective in reducing the rate of severe exacerbations by at least 63% in a study of budesonide and formoterol in combination[52] and by 47% in a study of fluticasone and salmeterol in a group of patients with moderately severe asthma.[3] The use of a combination of budesonide and formoterol as both maintenance and reliever treatment is superior to fixed-dose treatments in the reduction of the rate of exacerbations.[53,54] A more recent analysis[18] has defined a group of patients with persistent eosinophilic asthma on high doses of treatments as having a high exacerbation rate of 3·4 in the past year; exacerbations in this group of patients were reduced by roughly 50% after 1 year of treatment with the anti-interleukin-5 antibody, mepolizumab.[18] Other new treatments apart from combinations of inhaled corticosteroids and longacting β-agonists have also shown improvements in exacerbation rates in patients already on maximum asthma treatments. Those patients with allergic asthma on inhaled corticosteroid treated with the anti-immunoglobulin E antibody omalizumab were less likely to have an asthma exacerbation with a calculated odds ratio for five trials of 0·52.[55] With bronchial thermoplasty a 32% reduction was reported in the rate of severe exacerbations in the actively treated group compared with the sham-treated group with 0·48 versus 0·70 exacerbations per patient per year in patients already maintained on maximum doses of inhaled corticosteroid.[56] Finally, the longacting antimuscarinic agent tiotropium can also reduce the number of severe exacerbations in patients with severe asthma.[57]

Although these new treatments might have contributed to further reduction in the rate of exacerbations, further investigation of the potential heterogeneity of these exacerbations could dictate sensitivity to specific treatments. For example, the influence of the inflammatory phenotype on the type of asthma exacerbation is unclear. Most studies that have assessed the effect of a macrolide antibiotic in asthma have not shown any beneficial effect.[22,58,59] However, in the study by Brusselle and colleagues,[22] a predefined analysis confined to patients with a low blood eosinophil count as a marker of non-eosinophilic asthma, azithromycin, a macrolide antibiotic, was effective in reducing exacerbations.[22]

Corticosteroid insensitivity

Severe asthma is usually defined as inadequate symptom control despite the patient being treated with high doses of inhaled corticosteroid, often with oral corticosteroid treatment.[60] Oral corticosteroid-dependent asthma

represent individuals with asthma who need oral corticosteroids to control their asthma as shown by deterioration of asthma control on reduction or cessation of oral corticosteroid treatment. This category of patients can be considered as corticosteroid-insensitive.[61] Corticosteroid-resistant asthma is another term used to define a group of patients with asthma according to the response of FEV_1 after a 14 day course of oral prednisolone of 40 mg per day; those that respond by less than 15% increase in their pre-treatment FEV_1 are labelled as corticosteroid-resistant asthma, but they also needed to show a greater than 15% increase in FEV_1 to an inhaled β-adrenergic bronchodilator.[62] This test has yet to be validated as a marker of corticosteroid insensitivity.

Insensitivity to inhaled corticosteroid has been recognised by poor FEV_1 response,[63,64] and the T-helper-2-low phenotype measured in airway epithelial cells might be indicative of insensitivity to inhaled corticosteroid.[20] Increased sputum neutrophilia could also suggest corticosteroid insensitivity.[29] Individuals with asthma who smoke or who are obese are more likely to develop corticosteroid insensitivity.[65,66] In both instances, evidence exists of an important role for oxidative stress in underlying corticosteroid insensitivity.[67] Although a precise biomarker of corticosteroid insensitivity is not available, recent work on cells from patients with corticosteroid-dependent asthma has pointed to some potential mechanisms. Thus, several putative mechanisms for corticosteroid insensitivity have been proposed, and these might relate to specific phenotypes associated with severe corticosteroid-dependent asthma (figure 2). In severe asthma, data suggest that alveolar macrophages and airway smooth muscle cells are less sensitive to the effects of corticosteroids in inhibition of cytokine-induced release of chemokines such as CXCL8 and CCL11 compared with cells from patients with non-severe asthma.[68,69] Activation of p38 MAPK,[69,70] inability to recruit HDAC2 to the glucocorticoid receptor transcriptional complex,[71] reduced effectiveness of the ligand for glucocorticoid receptor binding,[72] an increase in the expression of the spliced variant of glucocorticoid receptor (glucocorticoid receptor-β),[73] and a deficiency in vitamin D3[74] have been proposed as different mechanisms of corticosteroid insensitivity.

Reversal of corticosteroid insensitivity by targeting any of these pathways might allow corticosteroids to work more efficiently, leading to improved asthma control at lower doses of inhaled or oral corticosteroid. Although this hypothesis remains to be tested in clinical trials, some insight has been obtained in the use of methotrexate and gold salts as a steroid-sparing agent in patients with steroid-dependent asthma.[75,76] In the case of methotrexate, some evidence exists to suggest that this treatment was associated with an improvement in circulating lymphocyte responsiveness to corticosteroids.[77]

New bronchodilators

Several once-daily $β_2$-agonists have been, or are being, developed that could lead to the use of once daily combination treatment with inhaled corticosteroid. This treatment would simplify asthma management and improve compliance with regular treatment. Once-daily $β_2$-agonists include indacaterol, carmoterol, milveterol, vilanterol, and olodaterol. Whether the use of a once daily combined treatment with inhaled corticosteroid and longacting β-adrenergic agonist would be more efficacious than a twice daily combination treatment is of interest.

The longacting muscarinic antagonist tiotropium has become a well-established bronchodilator for use in patients with chronic obstructive pulmonary disease, but only recently have trials been done in asthma. Tiotropium bromide improved lung function and symptoms in moderate-to-severe asthma patients who were not controlled on moderate-dose to high-dose inhaled corticosteroids with or without longacting β-adrenergic agonists.[78,79] In patients taking high doses of inhaled corticosteroids and longacting β-adrenergic agonists, the addition of tiotropium bromide improved FEV_1, reduced as-needed use of short-acting $β_2$-adrenergic agonists and

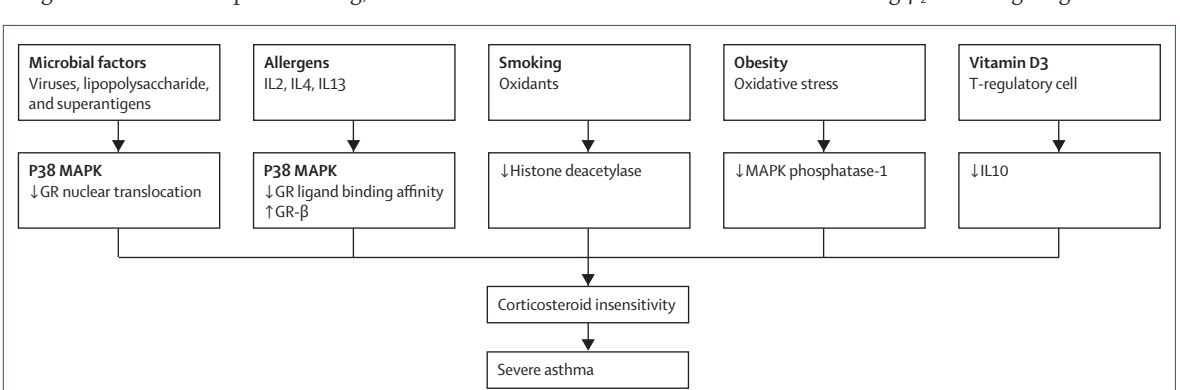

Figure 2: **Causes and putative molecular mechanisms of corticosteroid insensitivity underlying severe asthma**
IL=interleukin. MAPK=mitogen-activated protein kinase. GR=glucocorticoid receptor. T-reg=T-regulatory cell. Superantigens are products of bacteria or viruses that activate T cells.

modestly reduced the risk of severe exacerbation.[57,79] These results support the development of triple combination treatment with inhaled corticosteroids, longacting β-adrenergic agonist, and longacting muscarinic antagonist, or that of a combined longacting β-adrenergic agonist and longacting muscarinic antagonist or of a combined inhaled corticosteroid and longacting muscarinic antagonist. Triple treatment with a single inhaler containing a combination of inhaled corticosteroids, longacting β-adrenergic agonist, and longacting muscarinic antagonist might become standard treatment for severe asthma, on top of which additional novel treatments could be added.

Anti-immunoglobulin E monoclonal antibody

In the past 10 years, omalizumab has been the only new class of treatment introduced for the treatment of severe allergic asthma. Omalizumab is a humanised monoclonal antibody that binds to the high affinity immunoglobulin E receptor present on mast cells, basophils, and dendritic cells leading to a reduction in circulating immunoglobulin E, which prevents mast cells and basophils from releasing mediators when in contact with allergens. Findings from recent studies show that omalizumab might have airway anti-remodelling benefits by reduction of reticular basement membrane thickness in patients with asthma.[80,81] In patients with inadequately controlled severe persistent allergic asthma, despite high-dose inhaled corticosteroids and longacting β-adrenergic agonist treatment, and often additional treatment, omalizumab significantly reduced the rate of severe exacerbations and emergency visits, together with an improvement in asthma quality of life scores and improved symptom control.[82–84] Patients who are suitable for this treatment are given a trial of treatment for 4 months to assess therapeutic response before a decision is taken to continue treatment. Omalizumab can only be given to patients who are 6 years or older and who have a serum immunoglobulin E concentration of less than 1000 IU/L. More potent forms of anti-immunoglobulin E approaches that also have inhibitory activities on the synthesis of immunoglobulin E are being developed, and these might be used in patients with higher serum immunoglobulin E concentrations.[85,86]

Results from a retrospective analysis[87] showed that patients with a high amount of three biomarkers—FeNO, peripheral blood eosinophils, and serum periostin—showed the best improvement in terms of reduction in the number of exacerbations in response to omalizumab. Data from a small study suggests that omalizumab might also be beneficial in patients with non-allergic asthma, with a benefit in terms of improvement in FEV_1.[88] Therefore, use of serum immunoglobulin E might not be the best marker of response, and rather, markers associated with eosinophilic inflammation might be better alternatives.

T-helper-2 and other cytokines as novel targets

The T-helper-2 cytokine pathway has become one of the main targets for new treatments for asthma (figure 1) because T-helper-2 cytokines are expressed in the bronchial submucosa of patients with asthma. The pathway is characterised by a specific set of cytokines that include interleukin 4, interleukin 5, interleukin 9, and interleukin 13, which are released from T-helper-2 CD4 T cells and contribute to allergic airway inflammation. This process triggers the activation and recruitment of mast cells, eosinophils, and B cells that produce immunoglobulin E antibodies. A recent study of expression profiles of airway epithelial cells showed that not all asthma has a T-helper-2 signature,[25] and confirmed that individuals with a T-helper-2 signature have characteristics of an allergic inflammatory response.

T-helper-1 CD4 T cells are characterised by the production of interferon γ, which plays a part in dealing with intracellular infections, especially viruses, and in autoimmunity. Excessive T-helper-1 cell activation has been thought to be inhibitory of T-helper-2 cells, but more recently, has been shown to increase allergy and airway hyperresponsiveness in asthma. T-helper-17 cells mediate corticosteroid-resistant airway inflammation and airway hyperresponsiveness in mice,[89] and the T-helper-17 associated cytokines interleukin 17A, and interleukin 17F have been localised in the airways of patients with severe asthma.[37] Interleukin 17 might also be linked to neutrophilic asthma.[37]

Interleukin 33 and thymic stromal lymphopoietin are two newly discovered cytokines that could be regarded as future targets for novel treatments. Interleukin 33 is a member of the interleukin-1 cytokine family and an inducer and chemoattractant for T-helper-2 cells.[90] Expression of interleukin 33 is increased in the airway epithelium of patients with asthma.[91] Thymic stromal lymphopoietin is a cytokine related to interleukin 7 that is secreted by airway epithelial cells and which activates dendritic cells to release chemokines that are chemoattractant to and activate T-helper-2 cells.[92] The expression of thymic stromal lymphopoietin is increased in airway epithelium and lamina propria of patients with asthma, especially severe asthma.[93]

So far, most antibody approaches—apart from anti-immunoglobulin E—have been solely tested in adult patients with asthma. The fact that one study in children with severe asthma did not find substantial amounts of the T-helper-2 cytokines in bronchoalveolar lavage fluid[27] suggests that blocking these T-helper-2 cytokines might not be useful in children with severe asthma.

Anti-interleukin 4 approaches

Interleukin 4 is a T-helper-2 cytokine that has an important role in allergic airway inflammation through the activation of T-helper-2 cells, isotype class switching of B cells to immunoglobulin E synthesis, and in mast cell recruitment.[94] Interleukin 4 binds to interleukin-4

receptor α through two different types of receptors, type I and type II. Whereas type I receptors bind only to interleukin 4, type II receptors bind to both interleukin 4 and interleukin 13. Blocking type II receptors that are present on a wide range of cells leads to the inhibition of effects transduced by both interleukin 4 and interleukin 13. A study of pascolizumab, an anti-interleukin-4 humanised monoclonal antibody, in steroid-naive patients with asthma was stopped early because no evidence of clinical benefit was reported (NCT00024544). Interleukin 4 can be antagonised by the use of a recombinant human interleukin-4 receptor given in an inhaled form.[95] Findings from initial studies showed promise for the interleukin-4 receptor in the treatment of moderate persistent asthma,[96] but clinical trials have not shown any benefit in patients with mild asthma (NCT00001909). Pitrakinra, an interleukin-4 variant that binds to interleukin-4 receptor α and blocks the effect of both interleukin 4 and interleukin 13, reduced late phase allergen response in mild asthma.[92] In another study of pitrakinra of patients with asthma not adequately controlled with inhaled corticosteroids, no benefit of pitrakinra was reported (NCT00801853); however, a subanalysis of those with eosinophilic asthma showed a significant reduction in the number of asthma exacerbations at the highest dose of pitrakinra (NCT00801853). In a phase 2, randomised, double-blind, placebo-controlled study, AMG 317, a human monoclonal antibody to interleukin-4 receptor α that blocks both interleukin-4 and interleukin-13 pathways, did not show clinical efficacy in patients with moderate to severe asthma.[97]

Treatment with dupilumab, a human monoclonal antibody to the interleukin-4 receptor α subunit, was associated with reduced exacerbation rates when inhaled corticosteroids and longacting β-adrenergic agonist were withdrawn in patients with moderate to severe asthma with raised eosinophil concentrations.[21] Investigators also showed decreased serum concentrations of CCL26 (eotaxin-3), CCL17, and immunoglobulin E and concentrations of FeNO in exhaled breath, suggestive of suppression of T-helper-2 markers.[21] Findings from this study show that this new treatment could be used to replace inhaled corticosteroids and longacting β-adrenergic agonist combination treatment, leading to better control of asthma.

Anti-interleukin-5 antibody

Interleukin 5 is a T-helper-2 cytokine that is essential for the terminal differentiation, maturation, and survival of eosinophils. The anti-interleukin-5 antibody mepolizumab was not beneficial in unselected adult patients with moderate asthma,[98] but when studied in patients with severe asthma and persistent sputum eosinophilia, two anti-interleukin-5 antibodies, mepolizumab and reslizumab, decreased exacerbations and the use of oral corticosteroids, and improved symptoms and lung function.[17,18,99] A larger study[100] with mepolizumab showed efficacy in adults and adolescents in reduction of exacerbation rate, without improvement in FEV_1 and quality of life. Thus, mepolizumab should be targeted for patients with severe asthma with evidence of eosinophilic inflammation and a history of recurrent exacerbations.

MEDI-563 is a human monoclonal antibody directed against the interleukin-5 receptor α, which shows antibody-dependent cell cytotoxicity and dose-dependent effects in the reduction of the number of blood eosinophils.[101] A phase 2 study (NCT00768079) on the effect of MEDI-563 on exacerbation rates in patients who need an urgent health-care visit for treatment of an acute asthma exacerbation was completed in May 2011; no results have been published yet.

Anti-interleukin-9 antibody

Interleukin 9 has been linked to airway hyperresponsiveness, eosinophilic and lymphocytic inflammation; mast cell hyperplasia and interleukin-9 immunoreactive cells are increased in asthma. In a phase 2 safety study, MEDI-528, a humanised anti-interleukin-9 monoclonal antibody, was well tolerated with a non-significant lower number of exacerbations in the treated group of patients with mild to moderate asthma compared with placebo treatment.[102]

Anti-interleukin-13 antibody

Interleukin 13, together with interleukin 4, regulates immunoglobulin E synthesis and has an important role in mucus hyperplasia and airway hyperresponsiveness.[103] Interleukin 13 can also induce insensitivity to corticosteroids. Lebrikizumab, an antibody to interleukin 13, improved FEV_1 in adult patients with moderate to severe asthma, but did not affect exacerbations or asthma symptoms.[23] A beneficial effect was reported in patients with increased serum periostin concentrations, a proposed surrogate marker of T-helper-2 activity, or with raised concentrations of nitric oxide in the exhaled breath (FeNO). Another anti-interleukin-13 antibody, tralokinumab, did not improve symptoms but resulted in a non-significant increase in FEV_1 compared with placebo, with the highest increases in FEV_1 reported in those with detectable amounts of sputum interleukin 13.[104]

Chemoattractant homologous receptor expressed on T-helper-2 cell antagonists

The prostaglandin PGD2 activates a G-protein coupled receptor, chemoattractant homologous receptor expressed on T-helper-2 cells (CRTh2), also known as DP2 receptors, which are expressed on T-helper-2 cells and eosinophils. Activation of CRTh2 receptors on these cells leads to the chemotaxis of these cells.[105] Therefore, blocking the CRTh2 receptor might be beneficial in asthma, and CRTh2 antagonists are being developed for this reason. In individuals with moderate persistent asthma who are not on inhaled corticosteroids, a CRTh2

antagonist OC000459 improved FEV_1, asthma-related quality of life scores and night-time symptoms, without affecting sputum eosinophil counts.[106] In another study[107] of the CRTh2 antagonist AMG 853 as an add-on to inhaled corticosteroid treatment, no improvement in asthma symptoms or lung function in patients with inadequately controlled moderate-to-severe asthma was reported. It would be interesting to know whether applying this specifically targeted treatment for T-helper-2 high patients with severe asthma would lead to more effective control.

Tyrosine kinase inhibitor

Masitinib, a tyrosine kinase inhibitor that inhibits stem cell factor receptor (c-kit) and platelet-derived growth factor tyrosine kinase, improved asthma control in adults compared with placebo alongside a reduction in the dose of oral corticosteroids; however, no effect was reported on lung function.[108] Whether this treatment would be more effective in patients with evidence of high c-kit or tyrosine kinase activity is unclear; the best responders might be those with a mast cell component to their asthma.

Anti-CD25 antibody

Daclizumab, a humanised immunoglobulin G1 monoclonal antibody that binds specifically to the α (Tac, CD25) subunit of the high-affinity interleukin-2 receptor of activated lymphocytes and inhibits biological activity and binding to interleukin 2, improved FEV_1 and asthma control in adults with moderate to severe asthma that was inadequately controlled with inhaled corticosteroids.[109] Whether this treatment would work specifically in patients with evidence of underlying interleukin 2 or high T-helper-2 activity is unclear.

CXCR2 antagonist

CXCL8 (interleukin 8) might be an important chemokine involved in the chemoattraction and activation of neutrophils through the CXCR2 receptor, especially in severe asthma.[110] The CXCR2 antagonist SCH527123 inhibits ozone-induced airway neutrophilia measured in sputum samples in patients without asthma.[111] Treatment with SCH527123 also reduced sputum neutrophilia in people with severe asthma, and was associated with a modest reduction in mild exacerbations, but without an improvement in asthma control.[112] The definition of sputum neutrophilia in this study was taken as more than 40% of the differential sputum cell count. Inclusion of additional criteria to define the neutrophilic phenotype such as an eosinophil sputum count of less than 2% with a blood eosinophil count of less than 200μL could have led to better efficacy.

Anti-TNFα antibody

TNFα is a pro-inflammatory T-helper-1 cytokine that induces inflammation and hyperresponsiveness of the airway wall, mucus hypersecretion, and activation of macrophages. Golimumab, an anti-TNFα antibody, was ineffective in a study of adults with uncontrolled severe persistent asthma,[113] but data from a post-hoc analysis suggested that patients with a bronchodilator response of greater than 12% were less likely to experience severe asthma exacerbations while taking golimumab compared with placebo. However, further studies are unlikely to be done because of serious side-effects of golimumab, including an increased prevalence of infections in the actively treated group.[113] These findings are likely to be indicative of the importance of TNFα in controlling infections and possible malignancies. These results are in contrast to the established use of TNFα inhibitors in rheumatoid arthritis in which golimumab has been recommended in combination with methotrexate as an option for patients with severe active rheumatoid arthritis who have failed to respond to conventional antirheumatic drugs.[114]

Macrolide antibiotic treatment

Macrolide antibiotics have been used to treat asthma associated with *Chlamydia pneumoniae* or *Mycoplasma pneumoniae* infections. A trial[115] of roxithromycin in patients with asthma and serological evidence of infection with *C pneumoniae* did not improve asthma control. However, in a study of clarithromycin, an improvement in FEV_1 was only reported in patients with polymerase chain reaction-positive tissues for *C pneumoniae or M pneumoniae*.[116] In studies of paediatric asthma[53] and adult asthma,[59] the macrolide antibiotics, azithromycin and clarithromycin, respectively, had no effect on asthma control. Clarithromycin was used as an add-on treatment to inhaled corticosteroid in patients with severe asthma and reduced sputum neutrophils and concentrations of interleukin 8, together with an improvement in quality of life measures without changes in FEV_1.[117] However, in a study of mild-to-moderate persistent asthma that was suboptimally controlled by low-dose inhaled corticosteroids, the addition of clarithromycin did not further improve asthma control, although bronchial hyperresponsiveness was improved.[59] Finally, in the most recent study[22] of azithromycin in a cohort with severe asthma who were prone to exacerbations, azithromycin was associated with a significantly lower rate of severe exacerbations and lower respiratory tract infections requiring treatment with antibiotics than placebo in patients with non-eosinophilic (blood eosinophilia less than or equal to 200/μl) severe asthma. This effect was not recorded when the whole cohort was assessed together, although azithromycin significantly improved the Asthma Quality of Life Questionnaire (AQLQ) score overall.[22] This finding suggests that azithromycin could be beneficial in patients with non-eosinophilic severe asthma.

Macrolide antibiotics have also been tested as an adjunctive treatment to bronchodilators and corticosteroid treatments in patients with acute severe

exacerbations of asthma. A 10 day course of telithromycin improved asthma symptoms scores and symptom-free days, together with a greater improvement in FEV_1 by day 10 in comparison with placebo.[118] Whether types of asthma exacerbations exist in terms of causative factors and the inflammatory response—and therefore different responder profiles to antibiotic treatments—remains unclear. Investigators identified lower airway bacteria with a 16S ribosomal RNA technique and reported that patients with continuing asthma had markedly increased bacterial diversity and a greater bacterial burden compared with healthy controls without asthma.[119,120] Additionally, evidence suggests that phagocytosis of bacteria and apoptotic cells by macrophages might be impaired in patients with severe asthma.[34,121] The importance of these findings in relation to antibacterial treatments to improve asthma control needs to be determined.

Bronchial thermoplasty

Bronchial thermoplasty is a bronchoscopic procedure in which the large subsegmental airways are heated to 65°C by an electrode with radiofrequency energy. This usually needs three bronchoscopic procedures to treat all the large airways, and evidence suggests that this procedure leads to a reduction in airway smooth muscle mass at the site of thermoplasty. In a blinded, sham-control study,[56] bronchial thermoplasty reduced the number of severe asthma exacerbations, with a substantial improvement in asthma-specific quality of life with a reduction in days lost from school or work because of asthma. However, sham treatment also had a beneficial effect on quality of life measures, and 6% of patients undergoing the active procedure had to be hospitalised during the treatment period.[56] In an open study in patients with severe refractory asthma, thermoplasty improved FEV_1 and asthma control.[122] Which asthma phenotype would benefit most from this treatment is unclear, but if the mechanisms of its beneficial effect are attributable to a reduction in airway smooth muscle mass, patients with a continuing remodelling process might benefit the most.

Endotyping as a strategy for targeted treatments

Most studies of asthma treatments have characterised patients on the basis of asthma severity, but increasingly studies of potential new treatments are including criteria that better define asthma. Efficacy of new treatments will depend partly on the precision by which patients can be endotyped for the specific treatment being assessed.[123] Endotyping has been confined to measurements such as sputum eosinophils, exhaled breath markers such as nitric oxide, and mediators in blood such as serum periostin or blood eosinophils.[124] Biomarkers such as raised FeNO and serum immunoglobulin E seem to differentiate severe asthma from non-severe asthma in children but not in adults.[26] A recent expert group convened by the National Institutes of Health did a comprehensive search of the scientific literature and advised that only one measure—multiallergen screening—to define atopy be recommended as a core

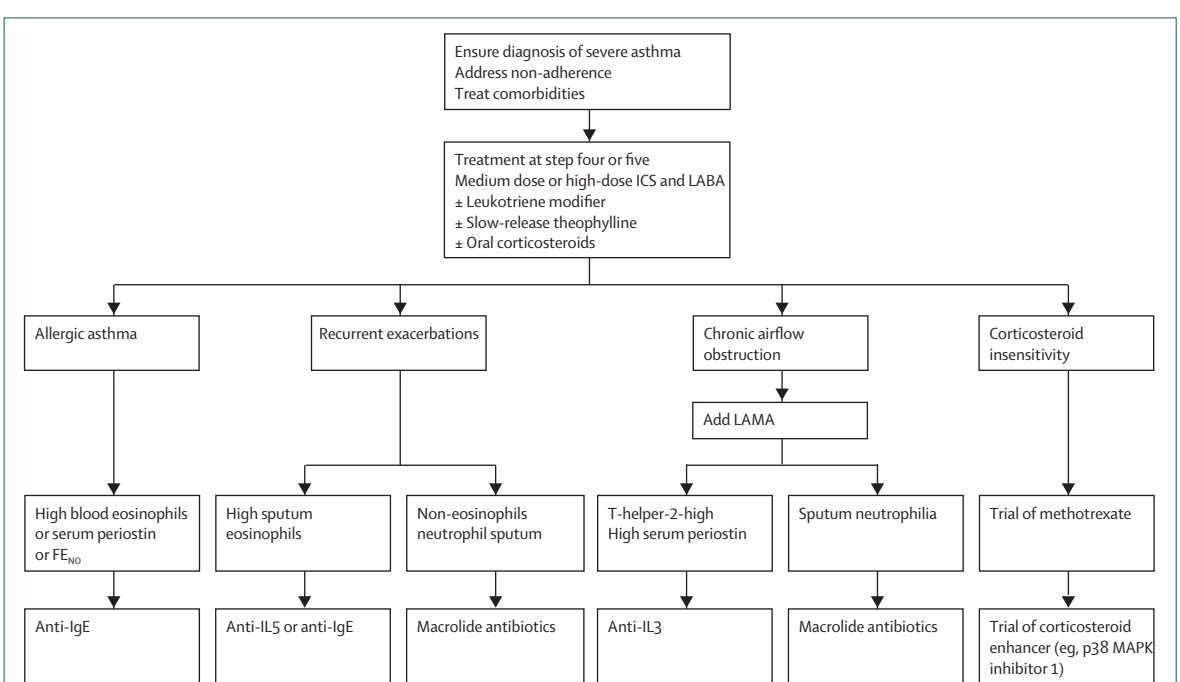

Figure 3: **Proposed management pathway with new treatments, focusing on characteristics and biomarkers**
ICS=inhaled corticosteroids. LABA=longacting β-adrenergic agonist. LAMA=longacting muscarinic antagonist. Fe_{NO}=nitric oxide in exhaled breath. IgE=immunoglobulin E. IL=interleukin. MAPK=mitogen-activated protein kinase.

asthma outcome.[125] Blood eosinophil counts, FeNO, sputum eosinophils, urinary leukotrienes, and total and allergen-specific immunoglobulin E were recommended as supplemental measures.[125]

FeNO could be used to detect eosinophilic airway inflammation, predict sensitivity to and need for corticosteroid treatment, and perhaps to detect non-adherence to corticosteroid treatment.[126] High concentrations of FeNO might predict patients who will respond best to anti-immunoglobulin E treatment in terms of reduction in exacerbation rates.[20] Moreover, high concentrations of FeNO identified those patients with severe asthma characterised by the highest amount of airflow obstruction and hyperinflation and most frequent use of emergency care.[127]

Sputum eosinophils and serum periostin can define a subset of patients who might respond well to some treatments such as the anti-T-helper-2 approaches with anti-interleukin-5 or anti-interleukin-13 antibodies. Use of the T-helper-2 signature from airway epithelial cells could be used to identify patients who would respond to treatment with inhaled corticosteroids; exhaled breath concentrations of nitric oxide could be used as a surrogate marker for therapeutic responsiveness to treatment with inhaled corticosteroids.[126] Serum periostin is a promising biomarker that could replace the use of epithelial cell expression of T-helper-2 cytokines, and is associated with airway eosinophilia.[48] The reduction in exacerbation rates as a result of treatment with omalizumab in severe allergic patients with asthma is best in patients with high concentrations of FeNO, blood eosinophilia, and serum periostin.[20] However, few promising biomarkers exist for non-eosinophilic patients and patients with asthma with low concentrations of T-helper-2 cells, which are usually associated with neutrophilic asthma and corticosteroid insensitivity. Only the low T-helper-2 endotype was a predictor of no or poor response to inhaled corticosteroids.[25] Patients with low eosinophil count and low FeNO concentration benefited from treatment with macrolides with a reduction in exacerbations and infective episodes of asthma.[22] Only a few genes that define the effect of asthma drugs have been identified so far.[128] More validated biomarkers in the endotyping of asthma are needed.

Systems biology approach

Many undiscovered mechanisms probably underlie the characteristics of severe asthma (panel). These characteristics might define the ultimate endotype of patients with severe asthma, who are in great need of new effective treatments. High-throughput biological data have improved understanding of the biological regulatory networks, which are made of proteins, RNA, and metabolites. Many researchers predict that the pathophysiological mechanisms underlying asthma are made up of different types of molecular and cellular components interacting through complex networks in various dynamic modes. These interactions will affect biological processes involved in inflammation, immunity, cell cycle, apoptosis, and metabolism, which will need to be linked to the clinical and phenotypic expression of asthma. The analysis of clinical, physiological, and high-throughput data from genomics, transcriptomic, lipidomic, and proteomic studies will provide a more complex and more accurate endotypic representation of individual patients. Additionally, epigenetic mechanisms such as DNA methylation, histone modifications, and microRNAs can modulate environmental effects, such as road traffic pollution and cigarette smoking, to affect the development and course of asthma, without alteration of nucleotide sequences.[129] This approach is being proposed in the Unbiased Biomarkers of Respiratory Diseases (UBIOPRED) project (funded by Innovative Medicines Initiative).[130] In this project, great efforts have been made to set up methods needed to apply systems biology to asthma. Analysis and processing of omics data have been organised with specific bioinformatics methods.[131] Many challenges need to be overcome including the complex multiscale biological organisation of molecular, genetic, proteomic, cellular, organ, and whole organism level data that need mathematical and computational methods for modelling pathophysiological and biochemical processes of asthma.[132,133]

Search strategy and selection criteria

The author searched PubMed for peer-reviewed research published in English between Jan 1, 2009, and June 1, 2013, using the search terms "new asthma treatments", "severe asthma", "biomarkers and asthma", and "anticytokine treatment". This search complemented the accumulated publications that the author has gathered with his involvement in asthma research and treatment during the past 20 years.

Present and future treatment strategies

Few targets exist that focus on the pathways associated with or dependence on T-helper-2 cells, and these pathways might constitute only a portion of patients with severe asthma. Although analysis of the characteristics of severe asthma suggest that other pathways should be considered, few targets are being investigated. Additionally, the high cost of drug development might result in investor reluctance to compounds that will only benefit a small group of highly phenotyped patients. One advantage of omics is the identification of more molecular targets for this disease. This new approach will change the business framework of drug development for asthma and other common complex diseases from the invention of blockbusters taken by all patients with asthma to the discovery of very specific drugs targeted at only a proportion of the asthma population.[134]

Because new treatments will be targeted at patients defined as having severe asthma, these patients should be

assessed first to confirm diagnosis, and to maximise the benefit of appropriate treatments. The management of non-adherence to treatments and comorbidities, such as gastro-oesophageal reflux, obstructive sleep apnoea, and rhinosinusitis, are very important aspects of the management of these patients. A stepwise increase in asthma treatments is the usual therapeutic approach advocated in asthma guidelines. The definition of severe asthma is dependent on non-response to the highest amounts of treatment given to patients at steps four and five of the GINA guidelines.[4,5] Any targeted treatments will probably be added to the treatment patients are already taking, usually high doses of inhaled corticosteroids and longacting β-adrenergic agonists with or without oral corticosteroids. In the future, biomarkers predicting response to treatments will form an important part of patient assessment so that appropriate targeted treatments can be recommended. Novel treatments such as anti-interleukin 5 might be beneficial in patients with evidence of eosinophilic inflammation usually established by the presence of sputum eosinophils of 3% or more. Because corticosteroids will remain the backbone of treatment for such patients, ways to improve the therapeutic effects of corticosteroids by reversing corticosteroid insensitivity should be investigated. Although it is too early to predict how our approach to management of patients with severe asthma with new treatments will evolve, figure 3 shows a potential approach based on the very scarce knowledge. Although this scheme cannot be used for current practice, the advantages of first establishing the most important characteristic of the patient with severe asthma and the restricted use of biomarkers predictive of treatment responsiveness are evident. Much more work is needed in terms of more precise and relevant endotyping of patients, and more targeted treatments are needed. The ability to endotype patients with severe asthma will allow for a more precise and rational way of getting these specific treatments to the individual patient; this will be the first step towards personalised medicine.[135] The challenge of delivering the benefits of personalised medicine to the patient remains high,[136] but this remains the only way that the right drugs will be delivered to the right patient.

Conflicts of interest

KFC has received honoraria for participating on Advisory Board Meetings discussing asthma and chronic obstructive pulmonary disease treatments with GlaxoSmithKline (UK), Astra Zeneca (UK), Merck (UK), Novartis (UK), and Gilead (USA), and has been on Speakers' Bureau of GlaxoSmithKline, Astra Zeneca, Merck, and Novartis. He has also received travel grants from Boehringer Ingelheim (UK) and Novartis to attend international respiratory meetings.

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